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Physiological and Biochemical Changes in Bacterial Cells Exposed to Oxygen

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Pure oxygen at 1 atm exerts two opposite effects on *Pseudomonas saccharophila*: it inhibits sucrose uptake and lipid synthesis, but it enhances the formation of polysaccharides.

Inhibition of protein synthesis in *Pseudomonas saccharophila* by 100% O_2 was reported previously (4). The effect of O_2 on synthesis of other cellular materials and on cell size in a resting state, with no net protein synthesis, are reported here.

Washed cells from 16-hr cultures were incubated in a nitrogen-free medium and gassed as described elsewhere (4). The change in optical density of cultures exposed to O_2 was considerably lower than that in cultures exposed to air (Table 1). The long and short axes of 100 distinctly separated cells of each of 3 types, taken randomly before and after incubation in air or in O_2 for 4 hr, were measured. The mean computed volumes, assuming a cylindrical shape, were 0.32 $\pm$ 0.001 μm^3 before incubation, 0.47 $\pm$ 0.01 μm^3 after 4 hr in O_2, and 0.69 $\pm$ 0.01 μm^3 after 4 hr in air. Neither the cell number nor the protein content of these cultures was changed after incubation for 4 hr in either O_2 or air.

In labeling experiments, the culture medium contained 0.2% nonlabeled sucrose and 0.015 μc of sucrose-U-^{14}C per ml. The results showed that the rate of ^{14}C accumulation in the cells exposed to O_2 was considerably lower than in those exposed to air (Table 1). The ^{14}C materials in the cells were extracted with methanol and chloroform (1). In the cells exposed to O_2, the radioactivity of the methanol-soluble fraction was 54% of that in cells exposed to air (Table 2). Four heavily labeled compounds, sucrose, glucose, fructose, and lipids, were identified in this fraction by means of two-dimensional chromatographic analysis. The chloroform fraction was characterized by the use of a silicic acid column (3); over 90% ^{14}C was found in glycolipids. The ratio of ^{14}C-chloroform-soluble substances in cells exposed to O_2 to those in cells exposed to air (0.46) was consistently lower than the corresponding ratio of total ^{14}C accumulation (0.55), which suggests some interference of lipid formation by 100% O_2.

Although O_2 depressed significantly the incorporation of ^{14}C-sucrose into methanol- and chloroform-soluble materials, it stimulated ^{14}C-sucrose incorporation into polysaccharides by 20 to 40% (Tables 1 and 2). Total polysaccharides of cells in other experiments after incubation for 4 hr were analyzed chemically (2); the polysaccharides increased 0.10 mg per mg of cell protein in cells exposed to air as compared to 0.18 mg in cells exposed to O_2 (*not shown in tables*).

To ascertain whether the increased formation of polysaccharides is a specific effect of high O_2 or a general phenomenon resulting from a reduced rate of cell enlargement, polysaccharide formation was studied in cultures incubated at a low temperature. Both the accumulation of ^{14}C and the change in optical density in the cells at 23 C were only two-thirds the corresponding values in the cells at 30 C (Table 3). In this case, however, ^{14}C-sucrose incorporated into polysaccharides was 50% lower in the cells at 23 C than in those at 30 C. These results provide evidence that the increased synthesis of polysaccharides is not a consequence of a reduced rate of cell enlargement.

The fact that 1 atm of oxygen stimulates the formation of polysaccharides from sucrose is surprising. Although the nature and cellular location of the newly formed polysaccharides are not clear, the accompanying inhibition of lipid synthesis suggests that the surfaces of cells exposed to O_2 may be somewhat different from those of cells exposed to air.

The present data show that O_2 at 1 atm exerts two opposite effects on nitrogen-deficient *P. saccharophila*. It inhibits cellular enlargement, accumulation of radioisotope, and the formation of lipids, but it enhances the formation of polysaccharides.

TABLE 1. *Accumulation of ^{14}C, formation of ^{14}C polysaccharides, and increase in optical density of nitrogen-deficient cultures exposed to air and to 100% O_2*[a]

Hour	Total ^{14}C accumulated[b]			^{14}C-polysaccharide formed[b]			Increase in optical density at 640 nm		
	Air	100% O_2	100% O_2/air	Air	100% O_2	100% O_2/air	Air	100% O_2	100% O_2/air
1	1,900	1,500	0.79	230	320	1.39	0.093	0.062	0.78
2	4,700	3,200	0.68	480	580	1.21	0.155	0.100	0.65
3	7,000	4,100	0.59	700	960	1.37	0.221	0.132	0.60
4	9,700	5,100	0.53	960	1350	1.40	0.256	0.150	0.59

[a] Culture medium contained 0.2% unlabeled sucrose and 0.015 μc of sucrose-U-^{14}C per ml (17,600 counts/min per ml). The cell concentration was 0.20 mg of cell protein per ml. Data on radioactivity studies represent the means of triplicate analyses on each of duplicate cultures of a typical experiment. The optical density values of cultures are the means of the duplicate cultures. The mean optical density values before incubation were 0.214 for air cultures and 0.220 for O_2 cultures.

[b] Values are expressed as counts/min per milligram of cell protein.

TABLE 2. *Radioactive compounds formed in nitrogen-deficient cells during 4-hr incubation in ^{14}C-sucrose*[a]

^{14}C-compounds	Air cells		O_2 cells		Activity in O_2 cells/activity in air cells
	Counts/min per mg of cell protein	Total ^{14}C accumulation	Counts/min per mg of cell protein	Total ^{14}C accumulation	
		%		%	
Total ^{14}C accumulation	171,200	100.0	94,610	100.0	0.55
Methanol-soluble substances	10,050	5.9	5,450	5.8	0.54
Chloroform-soluble substances	123,800	72.3	57,410	60.7	0.46
Polysaccharides	13,820	8.1	18,150	19.2	1.31
^{14}C-O_2 liberated	121,220		101,040		0.83

[a] Culture medium contained 0.2% unlabeled sucrose and 0.15 μc of sucrose-U-^{14}C per ml. The initial cell concentration was 0.33 mg of cell protein per ml. Data on radioactivity studies represent the means of triplicate analyses on each of duplicate cultures of a typical experiment. The amount of ^{14}C-O_2 liberated was computed from the decrease of total radioactivity in the cultures after incubation for 4 hr.

TABLE 3. *Accumulation of ^{14}C, formation of ^{14}C-polysaccharides, and increase in optical density of nitrogen-deficient cultures incubated at 23 and 30 C*[a]

Hour	Total ^{14}C accumulated[b]			^{14}C-polysaccharides formed[b]			Increase in optical density at 640 nm		
	23 C	30 C	23/30 C	23 C	30 C	23/30 C	23 C	30 C	23/30 C
1	1,865	3,349	0.56	92	170	0.54	0.050	0.105	0.48
2	4,067	6,978	0.58	238	472	0.50	0.109	0.209	0.52
3	4,783	8,963	0.65	411	663	0.62	0.163	0.269	0.61
4	7,724	11,341	0.68	468	852	0.56	0.213	0.324	0.66

[a] Culture medium contained 0.2% unlabeled sucrose and 0.015 μc of sucrose-U-^{14}C per ml. Two cultures were incubated at 23 C; another two cultures were incubated at 30 C. The initial cell concentration of each culture was 0.19 mg of cell protein per ml of culture; the optical density of cultures before incubation was 0.200. Data presented here were obtained in the same manner as those in Table 1.

[b] Values are expressed as counts/min per milligram of cell protein.

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